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FACTORS REGULATING THE DISCHARGE FREQUENCY IN OPTOMOTOR FIBRES OF *CARCINUS MAENAS*

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INTRODUCTION

An extensive study has been made of the optomotor system in *Carcinus* by Horridge with various collaborators (Barnes & Horridge, 1969*a*, *b*; Burrows & Horridge, 1968*a-c*; Horridge, 1966*a-e*, 1968; Horridge & Burrows, 1968*a-c*; Horridge & Sandeman, 1964). In these investigations many features were discovered by the use of various and ingenious techniques. The reason for the present paper and its sequel is that during a study of the responses from the optic nerve of this species (Wiersma, 1970), advantage was taken of the frequent occasions in which action potentials were obtained from optomotor fibres or muscles, and some additional facts were thereby discovered.

In the present paper we will deal especially with those influences which affect the discharge rates in various optomotor fibres. In the crayfish it has been noted that most motor fibres to the eye muscles are strongly influenced by the state of excitement shown by the animal (Wiersma & Oberjat, 1968). Though such an influence has been mentioned in passing by Burrows & Horridge (Burrows & Horridge, 1968*c*; Horridge, 1966*e*), it was not studied in any detail. It was found that an increase in the state of excitement in *Carcinus* leads also to increased discharges in most optomotor fibres, but that at least one specific motor neurone is inhibited by this state.

In addition it was observed that there was a pronounced change in habituation to constant stimuli when 'spring' and 'summer' animals were compared.

MATERIALS AND METHODS

Freshly caught animals were obtained from the Marine Station at Den Helder. They were kept at a constant temperature of 5 °C in a holding tank. Carapace width was about 5 cm. Preparations were made by fastening the animal with rubber bands to a metal plate, so that the body, legs and claws were pressed against the plate. Overt movements were thereby prevented, but during struggle reactions small body motions were still apparent. A small piece of the carapace next to one eyestalk was removed. This permitted free entry of the needle electrode through the soft membrane of the joint after the eyecup was fixed in its normal extended position by putting bonewax in the cavity behind it. This procedure prevented overt eye-withdrawal movements. Thin

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stainless steel pins, electrolytically and mechanically sharpened and coated with epoxy resin but for the tip, served as recording electrodes. The tips can advantageously be of a larger size (e.g. 5–10 μm) for the present purpose than for optic fibres (1–3 μm). The indifferent electrode was an ordinary uninsulated pin stuck through the carapace behind the brain region and connected to ground. The lead electrode was introduced through the soft membrane between the inner and outer eyestalk segments on the dorsal side, slightly posteriorly. The needle was usually pushed down in a medial direction, but other directions were also used to obtain different kinds of motor and muscle fibres.

The signals were fed into a Tektronix 122 pre-amplifier, and from its output into an audio system and an oscilloscope for visual display. They were permanently recorded with the aid of a Magnetophone 204 tape recorder. The records shown are replays from such tapes. Counts of the number of impulses per second were made mostly directly from the 'live' signals by an electronic counter, usually set for readings every 10 s with one or two s intervals.

RESULTS

In general, active leads from motor axons were obtained only from sites outside the main optic nerve. In a number of instances both motor nerve and muscle potentials were simultaneously present. In many cases, but not always, these showed a one-to-one relationship, and were therefore from the same motor system. Whereas the neural potentials were ordinarily constant in height when frequency changes occurred, the muscle potentials usually showed more or less pronounced increases in size with increased frequency. However, a decrease or increase in height also occurred sporadically, caused by a slight shift in needle position after changes in body position imposed to obtain other discharge frequencies. It was therefore necessary to judge in each instance whether or not a shift in position due to the weight of the needle played a role in the observed changes in size. In some instances intracellular muscle potentials were obtained as evidenced by the reversed polarity of the responses.

Optomotor fibres responsive to maintained body positions

The motor fibres which respond with a tonically maintained discharge proportionally to changes in body position are the most favourable for studying factors causing changes in their excitability. In the crayfish four of these are present, named according to the body positions which bring about their maximal firing rates: head-up, head-down, eye-up and eye-down fibres (Wiersma & Oberjat, 1968). To all indications these fibres innervate separate distinct muscles, which cause respectively a lowering, elevation, backward and forward rotation of the eyecup. In crabs, however, such selectivity may be absent, since Burrows (1967) has found that though a motor fibre innervates mainly one muscle, some fibres of adjoining muscles may also be innervated by it. Overlooking this possibility to a certain extent it is possible to ascribe to the motor fibres found in this work the functions performed by the following muscles assigned the similar task in *Carcinus* by Burrows & Horridge (1968*a*). The head-up fibres probably innervate muscle 18, with perhaps branches to 20*b*, which appears to assist in the movement; the head-down fibres may be specific for muscle 20*c*. The eye-up fibres are specific for 23*b*. They did not find muscles specific for the eye-down position, and we also failed to

find a motor fibre specific for this position. Only in a single instance among some 150 leads from motor fibres was there an indication of the presence of such a fibre. Also in agreement with their results was the finding of nerve fibres and muscle action potentials which reacted to changes of position along two axes instead of one, in several cases with no preferred reaction to one or the other. We thus found motor fibres which may be called head-up-eye-up fibres, which may specifically innervate their muscle 23*b*, as well as head-down-eye-down fibres, for which they describe two muscles, namely 19*b* and 22.

With regard to the horizontal optokinetic fibres, which we have called clockwise and anticlockwise fibres, the muscles involved according to Burrows & Horridge are 20*a* and 21. Here, however, upon rotation in one direction both will respond, one in either eye, so that the two nomenclatures cannot be reconciled. They are very good antagonists, so that the distinction is functionally of little importance in most aspects.

Table 1. *Head-up muscle fibre action potentials*

Impulses per 10 s in different positions 6–27

(90°)	(45°)	(0)	(30°)	(60°)	(45°)	(90°)
390	162	0	61	165	76	180
368	172	0	62	183	57	172
351	168	0	42	153	65	179
349	165	0	56	179	55	176
337	162	2	56	183	55	168
337	146	0	39	176	76	149
331	149	3	58	155	65	153
337	145	0	54	149	69	145
331	130	0	26	152	57	151

In each case there is evidence that during a unidirectional change in one axial plane three motor fibres may come into play: a pronouncedly tonic one, which appears always to be the smallest; a more phasic one, which reacts to position, but shows clear sign of preferred direction; and a still more phasic type, which is unidirectional. The latter type will be considered in more detail in the following paper. In any single lead any of the three types may occur separately or in combination. The medium-sized fibres show a more rapid increase in frequency when their maximal position is approached from the normal body position than when approached from the upside down position, but they will fire for either. As in the crayfish all tonic fibres, including the optokinetic ones, fire at a low rate in the normal body position, but are inhibited when turning takes place in the direction opposed to their preferred one.

For all the above-named fibres, the state of excitement of the animal, as indicated by its movements, is positively correlated with the firing frequency of the motor axon. Even when the body position is such that it provides for maximal inhibition, spikes can still be elicited when the animal is strongly excited by intense sensory stimulation. Changes in the excited state also occur spontaneously and then again its influence is pronounced or weak, correlated with the intensity of the movements of the appendages.

Fig. 1 shows the number of spikes counted over 10 s (with a 2 s interval between readings) for a protracted period in a tonic eye-up motor fibre. The counts were started 1 min after the animal was brought into this position. The interval permitted any possible increase in excited state, due to the rotation, to subside. There was usually a very slight downward trend in the frequency count which, however, became

only of significance over a period of an hour or more, and was interrupted by temporary increases due to increases in excited state. This very slow type of adaptation was found for all types of tonically reacting fibres in the late spring months. However, in the summer, adaptation became much more pronounced and more quickly established. This can be seen in Table 1 by comparing the readings at the same angle when repeated some minutes apart, e.g. at 45°. It could even lead in some instances to very

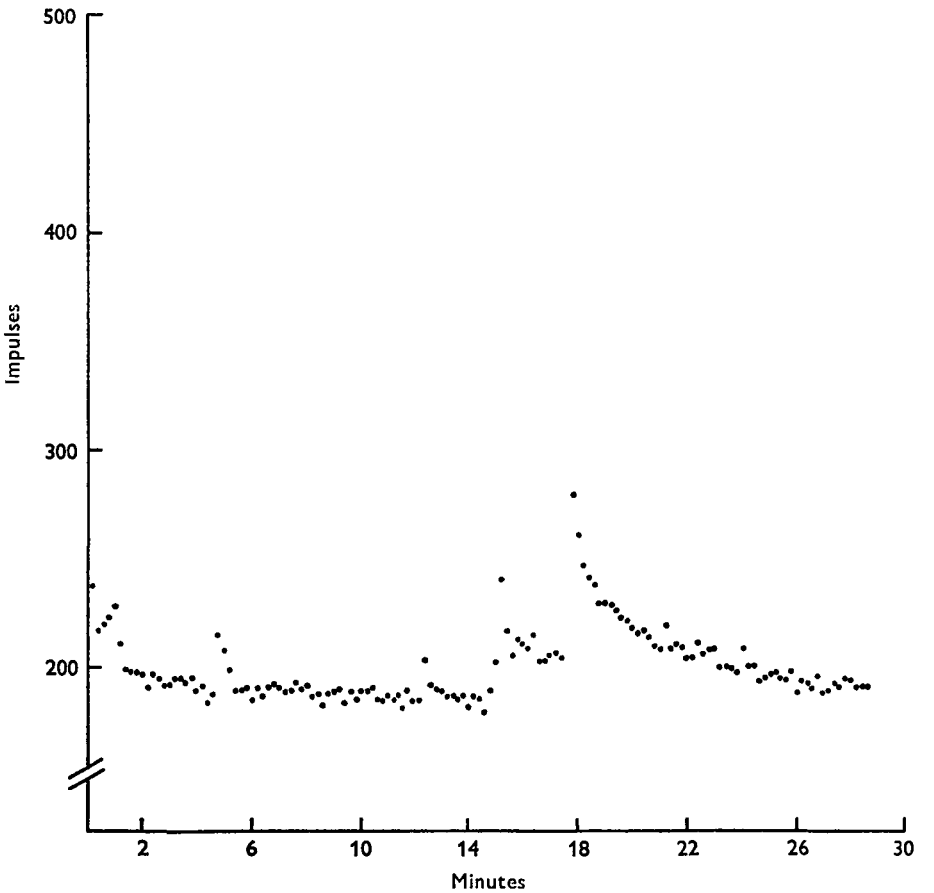

Fig. 1. Graph of the number of impulses in an eye-up motor fibre counted over periods of 10 s with 2 s intervals for reading. Total duration about ½ h., started 1 min after putting the animal in a 45° (thus not maximal) eye-up position. Note the constancy of the level, and several slight, and one very large increase in the number of spikes. These increases were correlated with changes in the excited state, as evidenced by the movements of the appendages and, at the large increase, also by body movements. Abscissa: minutes. Ordinate: impulses.

low impulse sequences in the preferred position for the medium-sized fibres unless an excited state occurred. This phenomenon also depended on the particular muscle or motor fibre used. Thus a pronounced difference was found between head-up and head-down fibres in these quickly adapting preparations. The head-down fibres usually still fired in the normal body position, whereas the head-up ones were silent; when a quiet animal was put in the head-up position the latter fibres could even adapt

completely after some period. In the spring data this difference was not obvious; both sets then showed a continuous discharge in the normal position, though that of the head-up fibres tended to be of lower frequency.

Table 2 shows measurements made during the summer on head-up–eye-up motor axons, and illustrates the fact that these fire strongly in both of these positions.

Table 2. *Comparison between two tonic motor fibres in indicated positions.* 8–12

Head-up	Eye-up	Horz.	Eye-down	Head-down
	Head-up-eye-up tonic nerve fibre			
64	59	19	7	0
63	61	17	9	0
62	59	17	7	0
60	53	15	8	0
62	59	14	10	0
60	56	16	10	0
	Pure eye-up tonic nerve fibre			
2	30	7	0	7
3	31	7	0	4
3	32	7	0	8
4	31	9	0	5
2	30	8	0	7
2	33	7	0	5

Impulses/s each mean 10 s

Optokinetic fibres

Horizontal optokinetic fibres, responding solely to rotation of the animal around the dorso-ventral body axis, were found to be also under the influence of the excited state, as mentioned also by Burrows & Horridge (1968*c*) and Horridge (1966*e*).

As in the crayfish, the crab has two sets of such fibres. It was found that in the latter the smallest tonic members of each set respond exclusively to visual input. In the next paper the more phasically reacting fibres will be analysed. These tonic fibres, one for clockwise rotations of the animal around the vertical axis the other for anticlockwise rotations, also show a resting frequency of a few impulses per second, and this frequency is markedly heightened in either fibre by an increase in excited state. During rotations in the null direction, when the resting discharge is inhibited, impulses will occur when the excited state reaches a certain level. In other respects our results were as expected, in that faster rotation speeds lead to faster increases in frequency, and that during a flipback inhibition followed by a renewed build-up takes place. All these findings are in accord with Horridge & Sandeman (1964). We also found that the flipback is often but not always accompanied or shortly preceded by a large eye withdrawal movement.

It is thus clear that all these optomotor muscles will be in a relatively greater state of tone when an excited state is present than in its absence. The same holds true for the discharges of the more phasic motor fibres and even for the unidirectional rotation fibres to be described in the next article. But for these latter very phasic fibres this is less easily demonstrated, since the quick motions necessary for their stimulation themselves tend to result in changes in the excited state.

Eye-withdrawal fibres

Both nerve and muscle potentials were obtained from the system which withdraws the eye backward into the socket in the carapace. Three motor fibres of this type were found. The very large phasic one which, according to Sandeman (1964, 1969) is located in the optic tract and has a diameter of 30–50 μm, is especially easy to obtain. As found previously, it shows quick habituation. In one respect, however, our observations on this unit differed from what has been previously reported regarding the quick eye-withdrawal reflex as such. It was observed that occasionally this fibre was triggered from areas far outside those described as its sensory field. Thus we have seen it fire on touch of the fifth contralateral leg. In general, it was noted that with increased distance from the experimental eye, habituation occurred more rapidly and lasted longer. For instance, repeated touch at one per second on the carapace above the eye might be effective only two or three times, stimulations of the antennule some ten times, whereas touch of the eye itself habituated only gradually and for a short time. Touch on very remote body parts, such as mentioned above, activate the fibre on only the first application and have such a prolonged refractory time that its duration could not be determined. Because of the pronounced effect of habituation it was not possible to decide whether or not the excited state has a definite influence on this reflex discharge.

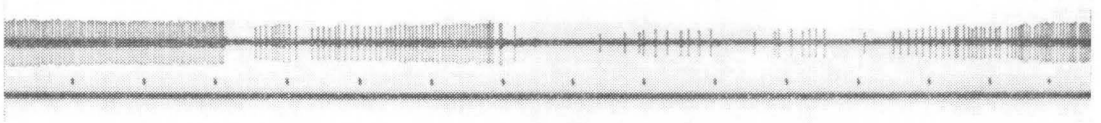

Fig. 2. Muscle action potentials from the tonic withdrawal muscle showing the influence of the excited state on frequency and size of the potentials. Time: 1 s.

Another phasically reacting fibre has already been described by Burrows (1967). In confirmation of his results its reponse was never elicited from remote parts of the body. When a combined lead was obtained, touch of the antenna caused a relatively weaker discharge in this fibre than in the large one whereas touch of the antennule was more effective for the smaller fibre. The discharges of neither of the fast fibres outlasted the stimulus duration to any extent. The third and much smaller fibre can keep the eye withdrawn for extensive periods of time. The chronic irritation caused by the needle or by the wax imbedding the eyecup would give rise to a rather regular discharge at, e.g. 35/s. These high rates are sensitive to the excited state which clearly had an inhibitory effect in every instance. Fig. 2 shows the muscle action potentials during a tonic withdrawal as well as the inhibiting influence of an increased excited state caused by stimulation of the legs. Quite clearly shown in the picture is the relationship between the height of the muscle action potentials and their frequency, as well as the inhibiting effect itself. In other instances, however, the action potentials showed no (or much less) difference in height, which may be ascribed to muscle fibres with differ-

ent amounts of facilitation, as are known to exist in other crab muscles (e.g. Atwood, 1967). It was found possible to make a crab extend a chronically withdrawn eye by bringing about a high state of excitement, with a return to the withdrawn position when this state subsided, showing that the inhibitory effect probably occurs as a behavioural response under natural conditions.

DISCUSSION

During these experiments there was again the absence of any indication of peripheral inhibition and of any feedback mechanism of eye position or movement on the motor discharges, in confirmation with the results of Horridge *et al.* on this crab and for the crayfish. As a consequence the optomotor system is more amenable to analysis than other crustacean reflex systems.

However, it is quite clear that in *Carcinus*, as in the crayfish, the impulse frequency to the optomotor muscles is greatly dependent on the momentary state of excitement of the animal, as is also indicated for other subsystems. It should be realized that the definition of this state is vague. From experiments with implanted electrodes in the crayfish it is known to be normally high under conditions of defence, and presumably also when struggling movements are being made. Normal movements are presumably accompanied by an increase, too, but there are reasons to believe that the increased reactivity of interneurones during nocturnal hours is not equivalent to the increase in excited state, as here considered. Increased responsiveness which is present in quiet animals during the night may depend more on hormonal than on neural factors, which are definitely the main basis for the influence of the excited state on interneurones and motor fibres (Aréchiga & Wiersma, 1969*a*, *b*).

That the effects of the excited state have not been especially noted in previous investigations of the optomotor system can be ascribed to the fact that the basic eye position as such will not be noticeably influenced by it, since all muscles responsible for the adjusting movements that were studied are equally affected by it. This is because, though the agonist in an adjusting motion will receive more impulses than in rest, so will its antagonist. In the experiments on motor neurone discharges (Burrows & Horridge, 1968*c*) where the factor was noted, its presence was undoubtedly minimized by the removal of the legs and the tight fastening of the preparation. A similar method has been used by Glantz (in press) to study the reactivity of sustaining fibres in the crayfish to light stimuli for protracted periods, and gave the same result, to wit, that spontaneously occurring changes in reactivity were minimized. With respect to the precision of induced changes in eye position under an excited state, it is unknown whether the accuracy would be greater, the same or less than in its absence. Theoretically the higher frequencies might provide an improvement since contraction levels may be smoother because of the greater number of impulses. It might, however, also lead to a faster jitter of the same or greater intensity than during rest.

The inhibitory influence of the excited state on the slow tonic withdrawal motor fibre is obviously of functional significance. It will put the eye in its normal working position when, because of an outside disturbance, there is need for maximal exposure of its receptive surface. It is, probably, only one of several inhibitory effects of the excited state. Taylor (1970) has, for example, found that an interneurone in the circumoesophageal commissure, which responds to quick motions of the statocyst, is

also inhibited by this state. However, there may be other complications, since he reports that this fibre 'anticipates' leg movements by an interval of up to a second or more, which indicates that the inhibition precedes the overt motions of the body. We have never seen any evidence of such long intervals with respect to either visual fibre reactions (e.g. Aréchiga & Wiersma, 1969*a*) or to optomotor fibre discharges. It seems certain that what is called, for convenience, an excited state, is not a single entity. Sandeman (1967) obtained, by electrical stimulation of the medial half of the ipsilateral esophageal commissure, inhibition of the withdrawal reflex which could be due to stimulation of the excited state's neural pathway.

The difference between spring and summer animals points to the influence of hormonal factors on mechanisms of synaptic transmission. The changes in reactivity appear to occur at about the time *Carcinus* migrates from deeper to shallower and more brackish water, but cannot be caused by that factor since our first group of animals showed it during the time they were kept in sea water of constant salinity. It appears to be a circannian type of change and deserves further study. There was some indication that by the end of August a reversal in the trend had occurred, but the experiments had to be stopped before this was clearly revealed.

A question presents itself about the functional significance of the increased tone in the majority of the optomotor muscles during the excited state. The joint between the inner and outer eyestalks is very flaccid, and the outer eyecup is known to sag when the blood pressure is too low to keep it 'blown-up'. Thus a resting discharge to all these muscles (but not to the eye-withdrawal ones) will normally prevent this sagging. From analogy with vertebrates one might expect that during the excited state the blood pressure would rise, and that therefore, if anything, fewer or no motor nerve discharges would be needed to keep the eyecup in place. However, there are indications that in crustaceans the heart rate and presumably the blood pressure fall during excited states by inhibition through the cardio-inhibitory nerves (Larimer, 1964). If this is normally the case the increased impulse frequency in all the nerve fibres involved would negate this effect. But it would also seem from the high frequencies elicited that an over-compensation takes place, so that additional factors may be involved. One might, in this regard, consider the fact that the excitability of many sensory neurones is greatly increased during an excited state. Therefore to obtain the same degree of movement of the eyecup on, e.g. an optokinetic stimulus, a greater resistance of the cup, caused by the muscular contractions, would be necessary to prevent an over-reaction. Further experimentation is obviously needed to solve these problems.

SUMMARY

1. The influence of the excited state of the animal on various motor neurone discharges and accompanying muscle action potentials was studied in the eyestalk of the crab, *Carcinus maenas*.

2. In most cases large increases in firing frequency could be obtained during such states. An exception is the tonic eye-withdrawal system in which an inhibitory effect is caused.

3. A pronounced difference in habituation to constant stimuli between spring and summer was found for the position fibres; in spring it was slow and in summer much quicker.

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